

**CONFIDENTIAL**

REPORT OF PROGRESS

December 5, 1955

to the

Tobacco Industry Research Committee

Period: March 1, 1955 - December 1, 1955

Title of Project: The production of genetically controlled animals and tumors for possible use by T.I.R.C. grantees in experimental research on tobacco in relation to health by (a) the expansion of known inbred stocks and sources of tumor supply; (b) the production of such hybrids or heterozygous types as become necessary; and (c) the relation of this material to specific experimental work at the Laboratory.

Name of Investigator: William S. Murray, Sc.D., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

During the period of this report there has been a marked increase in the number of research workers who have become converted to a realization of the benefits to be derived from the use of controlled animal material in their experimentation. The tendency has been for them to lean more and more heavily upon the material available at the Laboratory. This is particularly true of agencies undertaking contracts in the greatly expanded chemotherapy program of the National Cancer Institute.

The expansion undertaken by the Laboratory under the auspices of the Tobacco Industry Research Committee has proved to be very fortunately timed, in that it has made it possible to meet the increasing current demands of the period and lends itself to the much greater expansion demanded by the large testing programs planned by those working in chemotherapy.

The expansion within the Laboratory has been accomplished by (1) approximately doubling the size of the colony maintained in the nucleolus; (2) using the animals produced in this way to accomplish an expansion of approximately 15% in the size of the inbred nucleus, this 15% being confined to those of the inbred stocks currently in great demand; (3) the increased productivity of the nucleus

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has made it possible for us to stock the new animal room which was equipped under the non-recurring part of the budget of the T.I.R.C. grant.

The following table, which lists the number of animals produced in the new building, will give a picture of what we have been able to do in animal production through the support of this grant:

<u>MONTH</u>	<u>NUMBER OF MICE WEANED IN NEW BUILDING</u>
March	492
April	1122
May	2690
June	3462
July	3102
August	5246
September	3989
October	5688

This is over and above what we would have been able to do with our previous facilities.

At the present moment, the new building is operating at slightly over 50% capacity. We expect within the next year to bring it to capacity and, barring unforeseen difficulties, to produce in it animals at the rate of approximately 10,000 per month. Breeding animals on this scale makes it imperative that we continue to increase the diligence with which the animals in the nucleolus and in the nucleus are controlled, and that we make continuing studies of the various stocks in order to be able to insure the purity of strain. This is being done. In addition to this, we are also making studies of the normal behavior of a variety of first-generation hybrids, in an attempt to develop those types of animals for use in experiments to which they are adaptable.

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It has been our practice, in order to insure the purity of strain of the inbred animals sent to other laboratories and to minimize the chance of expanding sublines which vary from the standard set up for the parent strains, to confine animals sent from the Laboratory to those born in the first four generations after leaving the nucleus. The size of the nucleus thus becomes a controlling factor in the eventual number of inbred mice we are able to produce.

In some experiments, particularly those which use hosts for growing transplanted tumors, first generation hybrids have advantages over the parent stocks. Outstanding among these is the fact that many more of them can be produced within the limits of nucleus size and the control methods which we have established.

In the nucleus we are also maintaining 24 transplantable tumors of various types, are checking them frequently for the appearance of infection and taking immediate steps to clear up the contaminations which occasionally appear.

We have confidence that by continuing the program outlined above we shall be increasingly able to take care of any demands for animal material which researchers of the Tobacco Industry Research Committee may make upon us.

Of the possibilities for direct research within the Laboratory on problems of interest to the Tobacco Industry Research Committee mentioned in our proposal of December 15, 1954, we have been particularly intrigued by the influence of various carcinogens on transplants of cancer-susceptible and non-susceptible lung tissue. The techniques employed by Wynder and others in skin-painting of mice with tobacco derivatives and in transferring results thus obtained to a relationship with carcinogenicity of the lung, were considered.

It was felt that both the experimental setup and extension of the interpretation of results left much to be desired. Among the points which needed correction were the following:

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- (1) Environmental variation between the skin and the pleural cavity.

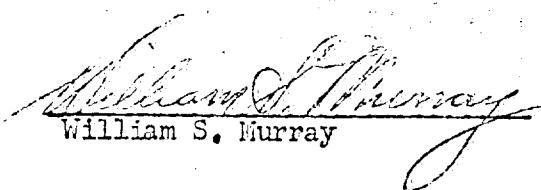
In order to approach more closely the temperature and other environmental conditions of the pleural cavity it was felt that the peritoneal cavity offered distinct advantages over the skin.

- (2) It was also felt that lung tissue itself should be challenged by direct or circulatory contact with the applied or infected material.

There is little structural or functional similarity between skin and lung.

- (3) It was also felt that equal opportunity should be provided in a single host animal for the two types of lung tissue (that giving a high natural incidence of adenocarcinomas and that giving relatively little incidence) to meet the challenge presented by transplantation to the peritoneal cavity.

The techniques for making all these improvements have been successfully developed and a considerable number of mice are already being challenged by known carcinogens as a precursor to later use of tobacco derivatives prepared under standardized conditions. (See Exhibit I).

  
William S. Murray

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## Exhibit I

## LUNG GRAFT - CARCINOGEN EXPERIMENT CENSUS AS OF 12/5/55

Group II	Donor	Route	Host	♀♀ BP*	♀♀ DBA**	♂♂ BP	♂♂ DBA	Total number mice	
1	BAF <sub>1</sub>	I.P. →	BAF <sub>1</sub>	--	--	--	--	--	208 I.P.
2	B/10	I.P. →	BAF <sub>1</sub>	13	--	19	--	32	
3	A	I.P. →	BAF <sub>1</sub>	32	23	27	21	103	
4	B/10	I.P. →	B/10	--	--	--	--	--	
5	A+B/10	I.P. →	BAF <sub>1</sub>	18	31	9	15	73	
6	BAF <sub>1</sub>	S.C. →	BAF <sub>1</sub>	10	--	10	--	20	324 S.C.
7	B/10	S.C. →	BAF <sub>1</sub>	22	28	9	30	89	
8	A	S.C. →	BAF <sub>1</sub>	14	66	13	35	128	
9	B/10	S.C. →	B/10	10	--	9	--	19	
10	A+B/10	S.C. →	BAF <sub>1</sub>	14	21	11	22	68	
								532	I.P. and S.C.

\* 3,4 - Benzpyrene I.V. (0.5mg/0.5cc/mouse) aqueous dispersion (W. E. Heston, N.C.I.).

\*\* 1,2,5,6 - Dibenanthracene I.V. (0.5 mg/0.5cc/mouse) aqueous dispersion (W.E.Heston, N.C.I.).

S.C.-Piece of lung placed S.C. through a small skin incision.

I.P.-Piece of lung sutured with absorbable catgut to the internal surface of the abdominal peritoneum.

Double grafts in one host (A+B/10): A lung placed on the left side and B/10 lung placed on the right side of the host.

Animals used: Inbred ♀ B/10 x ♂ A/Sn → BAF<sub>1</sub> offspring (age at graft: 5-7 weeks -- recorded)  
Inbred (brother x sister) pedigreed lines of A/Sn and C57Bl/10 ScBs  
maintained for the experiment

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